DYNAMICS OF PLAGUE RESISTANCE IN ALBINO MICE VACCINATED ONCE WITH FRACTION 1 OF *Pasteurella pestis*

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UDC 616.981.452-09-084.47:615.371:576.851.45/-07:616.981.452-097.5-078

A single injection of fraction 1 of *Pasteurella pestis* in Freund's complete adjuvant two days before infection with a virulent strain of this microorganism protected some albino mice from death.

The cyclic character of resistance to experimental plague and its intensity correlated with the dynamics of antibodies detected in the serum.

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The mechanism of immunity in plague and the role of individual antigens in the production of specific resistance have been studied intensively in many countries. Considerable progress has been made in this field as a result of work which has shown the nature and significance of the capsular substance, known as fraction 1 (F1). Evidence has been obtained that the preventive properties of the serum, detected by means of the passive mouse protection test, are due to the fact that it contains antibodies against F1. The resistance of immunized animals or animals surviving infection is also correlated with the level of F+ antibodies in their serum. Undoubtedly other antigens of *Pasteurella pestis* participate in the formation of antibacterial and antitoxic immunity. However, a high concentration of antibodies against F1 only provides resistance to large doses of the virulent microorganism.

The object of this investigation was to compare the dynamics of the serologic indices in albino mice vaccinated once with F1 and the dynamics of their resistance to experimental plague.

EXPERIMENTAL METHOD

A purified preparation of F1 was obtained from a saline extract of bacterial mass obtained by Baker's method by salting out with ammonium sulfate (0.3-0.33 saturation), followed by repeated reprecipitation. This preparation gave one precipitation line in gel against a plague agglutinating serum. The activity of F1 in the antibody neutralization reaction (ANR) was 0.001 μg/0.2 ml. The dose for immunization was 20 μg, or 20,000 minimal neutralizing doses (MND) per mouse. Antigen, made up in physiological saline or in Freund's complete adjuvant, was injected into the sole of the hind limbs in a dose of 0.05 ml, containing 10 μg F1, into each foot.

Serologic investigations were carried out with a stable diagnostic serum of high sensitivity – a 2.5% suspension of formalinized, tanninized sheep's erythrocytes, loaded with F1, in 10% formaline solution [1]. All the experiments were performed with series of the diagnostic serum of identical activity relative to agglutinating serum issued by the "Mikrob" Institute (Batch 69). The titer of this serum in the passive hemagglutination reaction (PHA) was 1 : 1,280,000.

The antigen and antibody concentrations were determined in the blood serum and saline extracts of the tissues from the place of injection of antigen. After blood had been taken, the mice were perfused with warm physiological saline (20-25 ml per animal) via the systemic circulation. The severed feet were weighed, ground up with a small amount of sand, and made into a 10% suspension. After centrifugation and heating to 56° for 30 min, the saline extract was filtered through paper and absorbed with sheep's erythrocytes. The sera absorbed by sheep's erythrocytes and the treated extracts were tested in the PHR and ANR.

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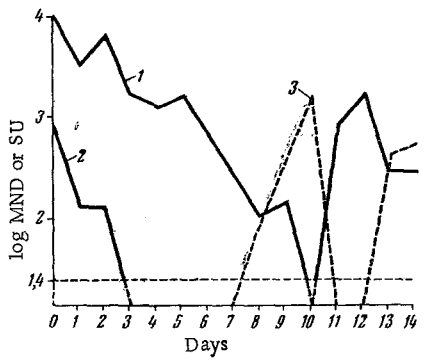

Fig. 1. Dynamics of antigen and antibody content in blood and tissue extracts from place of injection in mice vaccinated with F1 in adjuvant. 1) Curve of mean antigen concentrations in tissue extracts from place of injection (MND, minimal neutralizing doses); 2) the same in serum; 3) curve of mean antibody concentrations in serum (SU, serum units). Horizontal broken line, reference level.

The specificity of the results obtained in the PHR was verified by the hemagglutination inhibition reaction (HIR) with 8-16 MND of antigen.

The resistance of the albino mice was tested by infection with virulent strain No. 1300 of P. pestis. Uniformity of the conditions of infection was ensured by using one generation of microorganisms grown on solid medium with casein hydrolyzate. After growth for 48 h at 28°, the culture was kept in the cold throughout the experiment. Subcultures for infection were taken every two days and seeded on solid medium of the same batch. Tenfold dilutions of the 48-h culture of microorganisms were prepared in physiological saline. The number of living cells in the prepared suspensions was determined from the results of seeding on plates. Corresponding suspensions of microorganisms in a volume of 0.2 ml were injected subcutaneously into albino mice to produce infection.

The albino mice were immunized with F1 in Freund's complete adjuvant and in physiological saline (350 and 200 animals respectively). Corresponding groups of control animals were injected with adjuvant or physiological saline.

EXPERIMENTAL RESULTS

Of the group of animals vaccinated with F1 in adjuvant, 3 mice were sacrificed daily. The antigen content in their serum and tissue extracts from the place of injection are shown in Fig. 1. The curves are plotted from geometric mean titers. In this particular experiment F1 ceased to be detectable in the mouse serum after 2 days. On the 8th day antibodies were found, their titer reaching its highest level on the 10th day. However, neither on the 11th nor 12th day could antibodies be found in the serum. On the 13th day, they began to reappear. The dynamics of antigen detection in the tissues from the place of injection is of great interest. The high level of antigen in the extracts remained for the first 6 days after immunization. An appreciable decrease in its concentration coincided with the time of appearance of antibodies in the serum. In the period of the highest antibody level in the serum, the activity of the tissue extracts from the place of injection fell in the PHR to zero. The "disappearance" of antibodies from the serum coincided with a fresh "increase" in antigen concentration in the tissue extracts from the place of injection. The antibodies appearing on the 13th-14th day in the serum apparently lowered the antigen level at the place of injection.

The impression was gained that the dynamics of the antigen and antibody level in the blood and tissue extracts from the place of injection in mice immunized with a single dose of capsular antigen of P. pestis was cyclic in character. The same pattern was previously observed in other species of laboratory animals immunized with F1 in adjuvant (albino rats, guinea pigs, golden hamsters, and rabbits). A relationship was established between the species of animal, the dose of antigen, and the character of the cycles: the number and duration of waves and fluctuations in the antigen and antibody levels. Statistically significant results of experiments demonstrating the cyclic nature of the primary immunologic response in albino mice have been described in the literature [2]. The cyclic character of antigen and antibody dynamics also clearly reflected the dynamics of the plasma-cell response.

Alternation of periods when antigen was detected with "waves" of free antibodies evidently took place on account of the formation of antigen depots. The presence of antigen in the blood initially acted as a trigger for the mechanism of antibody formation and for the local response of hyperemia and inflammation. The antibodies which appeared formed neutral complexes with antigen liberated from the adjuvant. Only when one component (antigen or antibody) was present in excess could it be detected. Periods when neither antigen nor antibodies could be detected possibly developed when their proportions in the body were equivalent and when antigen was neutralized by antibodies.

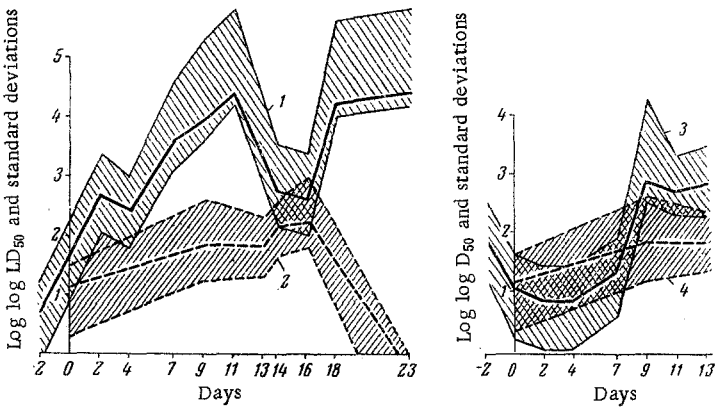

Fig. 2. Dynamics of resistance to infection with virulent strain of P. pestis. 1-4) Curve of log LD_{50} and standard deviations (P = 0.95; analysis by Kärber's formula) respectively for albino mice immunized with F1 + adjuvant, receiving adjuvant only, and immunized with F1 in physiological saline, and for control mice receiving physiological saline only. Abscissa: times between immunization and infection (in days).

The dynamics of resistance to experimental plague was the subject of a parallel study in equal groups of experimental (immunized) and control animals. The intervals between the infecting doses were short, 48-72 h. At each time groups of mice (6 for each dose) were infected with doses increasing tenfold from 1 to 10,000 bacterial cells. The results were subjected to statistical analysis. The level of resistance was expressed in log LD_{50}, and the standard deviations were calculated by Ashmarin's method. It is clear from Fig. 2 that injection of 20 μg F1 in physiological saline increased resistance to infection very slightly and relatively late – 9-13 days after immunization. However, the same dose of antigen in adjuvant modified the sensitivity of the animals to the virulent strain. The mice of the experimental groups 2 days after immunization were more highly protected than the controls receiving adjuvant without antigen. Resistance increased until the 11th day. On the 14th-16th the resistance of the immunized animals fell to the same level as that of the control mice. When immunity was tested at the subsequent periods, considerable resistance of the immunized mice was again found, distinguishing them essentially from the controls.

Comparison of Figs. 1 and 2 shows that the curves of resistance and of antigen and antibody dynamics are connected. An increase in resistance to infection at successive periods coincides with the periods of increase in antibody titers. The decrease in resistance observed on the 14th-16th day after injection of F1 + adjuvant preceded a second wave of antibodies in the serum.

The early resistance observed soon after injection of F1 + adjuvant had no apparent explanation: Antibodies in detectable amounts were recorded only after the 8th day. No antibodies were found in mice receiving F1 without adjuvant, using serologic reactions and a phagocytic test. Very probably in the early period after immunization the antibodies possessed little avidity, they were present in small amounts, and were quickly used up by fixation with the microorganisms. The degree of resistance in the early stages was much lower than the level of protection afforded to the animals subsequently, when it rose parallel with the rise in antibody titer. It has been shown that early protection of albino mice immunized with F1 is specific: The animals resisted experimental plague but not tularemia or melioidosis [3, 4]. Resistance to plague antigens of early onset has also been described in cold-blooded animals and even in insects. However, it is of low specificity and short duration. It may be assumed that the phenomenon of early protection also persisted in the case of more highly organized animals, but in the course of their evolution it became specialized and a more specific primary inmunologic response appeared.

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